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Evaluation of Polycyclic Aromatic Hydrocarbons Using Analytical Methods, Toxicology, and Risk Assessment Research: Seafood Safety after a Petroleum Spill as an Example

Jeffrey Wickliffe,[1] Edward Overton,[2] Scott Frickel,[3] Jessi Howard,[1] Mark Wilson,[1] Bridget Simon,[1] Stephen Echsner,[1] Daniel Nguyen,[4] David Gauthe,[5] Diane Blake,[1] Charles Miller,[1] Cornelis Elferink,[6] Shakeel Ansari,[7] Harshica Fernando,[7] Edward Trapido,[8] and Andrew Kane[9]

[1]Department of Global Environmental Health Sciences, Tulane University, New Orleans, Louisiana, USA

[2]Department of Environmental Sciences, Louisiana State University, Baton Rouge, Louisiana, USA

[3]Department of Sociology, Washington State University, Pullman, Washington, USA

[4]Mary Queen of Vietnam Community Development Corporation, Inc., New Orleans, Louisiana, USA

[5]Bayou Interfaith Shared Community Organizing, Thibodaux, Louisiana, USA

[6]Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas, USA

[7]Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, Texas, USA

[8]Department of Epidemiology, Louisiana State University Health Sciences Center, New Orleans, Louisiana, USA

[9]Department of Environmental and Global Health, University of Florida, Gainesville, Florida, USA

Address correspondence to:

Jeffrey Wickliffe

Address-Department of Global Environmental Health Sciences, 1440 Canal Street Suite 2100,

Tulane University, New Orleans, LA 70112

Telephone-504-988-3910

E-mail address-jwicklif@tulane.edu

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Abstract

Background: Polycyclic aromatic hydrocarbons (PAHs) are abundant and widespread environmental chemicals. They are produced naturally and through man-made processes. They are common in organic media including petroleum. Several PAHs are toxic and a subset exhibit carcinogenic activity. PAHs represent a range of chemical structures based on 2 or more benzene rings and depending on their source can exhibit a variety of side modifications resulting from oxygenation, nitrogenation, and alkylation.

Objectives: Highlight the increasing ability of contemporary analytical methods to distinguish not only different chemical structures among PAHs but also their concentrations in environmental media. Describe the emerging issues in the PAH risk assessment process due to increasing analytical sensitivity for individual PAHs and the paucity of toxicological literature for many of these compounds using seafood contamination following the Deepwater Horizon accident as an example.

Discussion: PAHs, including the large variety of chemically modified or substituted PAHs, are naturally occurring and may constitute health risks if human populations are exposed to hazardous levels. However, toxicity evaluations have not kept pace with modern analytic methods and their increased ability to detect substituted PAHs. Therefore, although it is possible to measure these compounds in seafood and other media, we do not have sufficient information on the potential toxicity of these compounds to incorporate them into human health risk assessments and characterizations.

Conclusions: Future research efforts should strategically attempt to fill this toxicological knowledge gap so human health risk assessments of PAHs in environmental media or food can be better determined. This is especially important in the aftermath of petroleum spills.

Introduction

In the past several decades, there have been numerous petroleum leaks from transport vessels, pipelines, and exploration wells. Production well accidents have also resulted in several large spills. Natural seepage of crude oil also contributes to the petroleum load in the environment (Farwell et al. 2009). Spills and leaks in coastal areas and adjacent marine environments can negatively impact marine and coastal biota and increase concern regarding potential health effects in cleanup workers and residents. Contamination of locally harvested seafood species with toxic polycyclic aromatic hydrocarbons (PAHs) represents a major concern as consumption is a major route for human exposure (Dickey 2012; Gohlke et al. 2011; Goldstein et al. 2011; Peacock and Field 2009; Rotkin-Ellman et al. 2011; Rotkin-Ellman and Solomon 2012; Xia et al. 2012).

With respect to seafood caught or harvested in spill or contaminated areas, subsistence, recreational, and commercial fisheries are invariably impacted. Local, state, and federal public health officials are charged with monitoring seafood safety during and following petroleum accidents to minimize possible health effects that may result from consumption of contaminated seafood. Researchers typically measure petroleum contaminants in potentially impacted coastal and marine species, including seafood, as a component of their research to estimate possible ecological or human health consequences. Debate regarding seafood safety and estimates of possible increased health risks during and following such events has often centered on key aspects of health risk models and the health risk assessment process (Dickey 2012; Gohlke et al. 2011; Rotkin-Ellman et al. 2011; Rotkin-Ellman and Solomon 2012). For example, health risk-based levels of concern for PAHs in seafood calculated by the United States Food and Drug Administration (FDA) following the Deepwater Horizon accident have been questioned as not

protecting sensitive subgroups such as the developing fetus, children, and women primarily of childbearing age (Dickey 2012; Gohlke et al. 2011; Rotkin-Ellman et al. 2011; Rotkin-Ellman and Solomon 2012). In fact, little is known in the field of toxicology regarding the negative health effects that consumption of PAHs especially from consumption of contaminated seafood may have on the developing fetus, children, or adolescents. Furthermore, application of various acceptable risk levels, consumption rates, exposure duration, and estimates of body weights have been hotly debated as these metrics, in addition to measured PAH levels, drive the estimates of both cancer and non-cancer disease risks (Dickey 2012; Gohlke et al. 2011; Rotkin-Ellman et al. 2011; Rotkin-Ellman and Solomon 2012).

Polycyclic aromatic hydrocarbons (PAHs) primarily consist of carbon and hydrogen. PAHs have two primary sources: they are formed either by combustion of organic matter (e.g. forest fires, fossil fuel combustion) or by the diagenetic transformation of mostly plant material deposited deep within the earth's crust. Thus, the two sources of PAHs are known either as pyrogenic or petrogenic, respectively. These formative processes do not produce a single molecular structure but instead generate cyclic compounds with 2 to 7 fused benzene rings of different shapes and sizes. There are hundreds of different PAHs; however, the composition of PAHs from combustion is noticeably different than that produced by diagenetic processes (PAHs found in crude oil, coal, shale etc). Pyrogenically produced PAHs consist primarily of unsubstituted aromatic ring compounds often called parent PAH structures. Petrogenically-produced PAH compounds have alkyl group substitutions on the various parent ring structures. A minor fraction of the PAHs found in petrogenic sources include unsubstituted or parent compounds. Therefore, analytical data that includes information on alkyl substitution makes it relatively easy to determine if the PAHs in an environmental sample are from pyrogenic or petrogenic sources, or

a mixture of both. Our objectives are to highlight the increasing ability of contemporary analytical methods to distinguish not only different chemical structures among PAHs but also their concentrations in environmental media and to describe the emerging issues in the PAH risk assessment process due to increasing analytical sensitivity for individual PAHs and the paucity of toxicological literature for many of these compounds using seafood contamination following the Deepwater Horizon accident as an example.

Discussion

Analytical chemical methods designed to determine the compositional nature and quantity of specific PAHs in environmental and biological media have progressed substantially in the last ten years. Contemporary methods using gas chromatography (GC) followed by mass spectrometry (MS) under selective ion monitoring (SIM) modes can now discriminate hundreds of specific PAH compounds as well as their individual quantities in complex biological samples. For example, advanced methods using highly automated and efficient extraction protocols coupled with GC and quadrupole MS rapidly identify and quantify classic unsubstituted PAH analytes (UPAHs, e.g. benzo[a]pyrene, chrysene and naphthalene) as well as historically underrepresented nitrogenated (NPAHs), oxygenated (OPAHs), and alkylated (APAHs) homologs (primarily in the C_{10}-C_{25} range). Method detection limits (MDLs) have also improved considerably such that levels of unsubstituted and substituted PAHs can now be determined in the low parts per billion to high parts per trillion (Gohlke et al. 2011, Overton et al. 2004, Rotkin-Ellman et al. 2011, Xia et al. 2012). These values are often 1-3 orders of magnitude lower than levels of health concern for the small subset of PAHs for which we have adequate toxicological and health risk information.

UPAHs such as benzo[a]pyrene are primarily produced through incomplete combustion or the pyrolysis of organic material including fossil fuels. NPAHs and OPAHs are produced through combustion of fossil fuels, atmospheric processes, and microbial and enzymatic activity (Albinet et al. 2007; Durant et al. 1996; Lundstedt et al. 2007). APAHs are generally found in relatively high concentrations as native constituents in crude oil or petroleum and have been used as petrogenic biomarkers to identify unrefined, uncombusted petroleum in the environment (Overton et al. 2004; Saha et al. 2009). Overton et al. (2004) used GC/MS-SIM to apportion PAH sources in coastal, marine sediments as a function of historical and contemporary oil and gas activity in the Gulf of Mexico, categorically discriminating the pyrogenic PAHs (e.g. naphthalene) from the petrogenic PAHs (e.g. methylnaphthalenes).

What is known about the toxicology of several of the UPAHs is extensive. Within the class of PAH compounds, there are both non-carcinogens and carcinogens. In general, the small PAHs (2- to 3-ring members) act as non-carcinogens mainly affecting the respiratory, neurological, or immune system. Some of the smaller PAHs may act as comparatively weak carcinogens at high concentrations. The larger PAHs (4- to 7-ring members) may also act as non-carcinogens (e.g. immuntoxic) but primarily act through a mutagenic mode of action as fairly potent carcinogens (ATSDR 1995, IARC 1983, NTP 2011). Based on current knowledge, the concentrations or levels of the various PAHs in environmental media including seafood associated with either non-cancer health effects or cancer vary. The levels of concern (LOCs) for PAHs that may cause non-cancer health effects are considerably higher than those LOCs that are linked to cancer (Gohlke et al. 2011, Rotkin-Ellman et al. 2011). For example, in shrimp or other orally consumed media the LOCs (i.e. those at which an adverse health effect for a fraction of the exposed population may be expected) for non-cancer health effects expected from PAHs (naphthalene, anthracene, 2-

methylnaphthalene, and acenaphthene) ranges from low to high ppm levels (Gohlke et al. 2011, Rotkin-Ellman et al. 2011). In contrast, for cancer effects expected from the PAH benzo[a]pyrene, the LOC is in the low ppb range (Gohlke et al. 2011, Rotkin-Ellman et al. 2011).

The levels of PAHs detected in seafood and many other environmental media are often low (not detected to low ppb) and are either below or near the LOCs for cancer health risks (Gohlke et al. 2011, Rotkin-Ellman et al. 2011, Xia et al. 2012). There are some food items (e.g. smoked foods) that have comparatively high levels of PAHs, mostly higher molecular weight compounds, on their surface, and these may well represent both non-cancer as well as cancer risks (Silva et al. 2011, Stolyhwo and Sikorski 2005). As discussed more fully below, PAHs in seafood tested during and following the DWH event up to the present day were at or below the LOCs for cancer health risks and far below those associated with non-cancer health risks. This is the case for PAHs found in most environmental media including foodstuffs. Thus, the primary health concern based on the toxicological evidence to date when considering the vast majority of human population exposures to PAHs in environmental media is cancer. Support for cancer as the primary health concern following the DWH event and possible contamination of seafood can be found in several recent publications (Dickey 2012; Gohlke et al. 2011; Rotkin-Ellman et al. 2011; Rotkin-Ellman and Solomon 2012; Xia et al. 2012). Therefore, we will frame this commentary from the perspective of carcinogenesis.

Unsubstituted PAHs generally require enzymatic bioactivation to highly reactive compounds that covalently modify DNA, forming premutagenic DNA lesions or adducts (Klaunig and Kamedulis 2008; Shimada et al. 2004). A few of the NPAHs and OPAHs have also been evaluated for mutagenic potency, and many of these exhibit genotoxicity with or without

enzymatic activation (Durant et al. 1996). For the UPAHs, there is considerable heterogeneity in mutagenic and hence carcinogenic potency (Collins et al. 1998; Nisbet and LaGoy 1992; EPA 2000, EPA 2010). This is thought to derive from the structural properties of the various UPAHs. Genotoxicity appears to be a function of bay or fjord configurations in which the presence and size of the configuration influences the detoxification efficiency of the bioactivated metabolites. For example, naphthalene is relatively non-genotoxic and has neither a bay or fjord configuration or moiety. On the other hand, benzo[a]pyrene and dibenzo[a,i]pyrene are genotoxic and carcinogenic UPAHs with different individual potencies that harbor a bay and fjord region respectively.

The levels of UPAHs in environmental media and food are monitored by federal health agencies including the United States Evironmental Protection Agency, FDA, and National Oceanic and Atmospheric Administration. However, the UPAHs comprise a relatively minor fraction of the total number and mass of PAHs found in crude oil and crude oil-contaminated seafood, as compared to the APAHs (Saha et al. 2009). Xia et al. (2012) examined seafood from areas impacted by the DWH event for PAHs including APAHs. They found that low molecular weight UPAHs and several APAHs were present in most seafood types examined (Xia et al. 2012). UPAHs used in health risk assessments conducted by the FDA and others were generally low or below the detection limits in their study (Dickey 2012, Rotkin-Ellman et al. 2011, Xia et al. 2012). They also noted higher levels of total PAHs during July-October 2010, that fell to much lower levels by early 2011 (Xia et al. 2012). It is plausible that APAHs were in some measure responsible for the higher levels of total PAHs during the spill. It is therefore unfortunate that comparatively few of the APAHs have been evaluated for toxicity or mutagenicity. Those that have been evaluated suggest reasons for concern. For example, past research has demonstrated

that 5-methylchrysene is significantly more toxic and carcinogenic than the unsubstituted, parent chrysene (Hecht et al. 1978). The lack of toxicological data and related risk information on the APAHs represents an especially critical gap in the scientific data because by mass the APAHs constitute the vast majority of PAHs in the crude oil and petroleum with the potential to contaminate seafood following a marine spill event (Baird et al. 2007).

For these reasons, an important opportunity exists for narrowing the knowledge gap between what researchers currently know about specific types and levels of PAHs in seafood and how that information can be used for subsequent estimates of possible increased health risks following consumption. The EPA currently includes 16 UPAHs in the analysis of environmental media (i.e. soil/sediment, water, or air) for protecting public health (Nisbet and LaGoy 1992; Schoeny and Poirier 1993). Seven of these UPAHs are considered the key carcinogens for the purposes of policy-based cancer risk assessments (EPA 2000, Schoeny and Poirier 1993). With the exception of benzo[a]pyrene, oral slope factors for estimating cancer risks at the federal level are unavailable for most of these recognized carcinogens. Therefore, researchers and the EPA have developed and applied relative potency factors (RPFs) designed to scale carcinogenic potencies to benzo[a]pyrene assuming simple additive toxicity (Collins et al. 1998; EPA 2000; EPA 2010; Nisbet and LaGoy 1992). The FDA and NOAA risk assessment protocols include the EPA 16 as well as 9 additional PAHs including a select few APAHs (FDA 2010). These protocols are designed to provide quantitative measurements for assessment of ingestion health risks and inform any risk management strategies or consumption advisories that may be warranted. In addition, the FDA and NOAA protocols can provide limited information on the source of the PAHs (i.e. pyrogenic or petrogenic). Research scientists currently funded to conduct seafood safety assessments in response to the Deepwater Horizon accident are now

including an additional 25-50 PAHs, most of which are APAHs, to better define the pyro- versus petro-genic origin of these compounds in seafood and marine species. These consortia, with whom many of us are working, are in the process of collecting and analyzing seafood and as such that scientific data has not yet been published. Thus this emerging gap in risk assessment is driven by 1) the contemporary technological capacity to measure an array of PAHs at extremely low concentrations, and 2) the general absence of toxicological information for many PAHs and virtually all of the APAHs regarding toxicity, mutagenicity, or carcinogenicity. Closing this gap should be a pressing concern for scientists, risk assessors, and public health officials. We argue that our ability to quantify 50-100s of PAHs, while having meaningful information on human health risk for a small fraction of these compounds, is a major problem for conducting risk assessments and accurately communicating risks regarding seafood safety as well as other situations in which substituted PAHs represent either consumption or inhalation risks.

Our capacity to detect smaller and smaller quantities of PAHs in seafood and other matrices could lead to misperceptions by the public about the health risks that they may face after oils spills and natural disasters. Risk assessments often treat non-detects or levels below the MDL as zero when evaluating health risks. The lay public also may interpret such non-detect or sub-MDL findings inseafood samples as indicating that they are essentially free of PAHs. Today however, many more PAH analytes can be assigned quantitative values in complex environmental media, like seafood, that may have previously been considered PAH-free. This means that analytes with quantitative values above the currently available MDLs (low ppb/high ppt) can be used in mixtures risk assessment models, even if individual PAHs are present in concentrations far below currently accepted levels of health concern. This new analytical capacity may trigger health concerns among members of the general public, for whom the mere detection of PAHs in

seafood may be interpreted as evidence of a problem. Adding complexity to this emerging problem is the relative absence of a comprehensive evidence base by which the toxicity, mutagenicity, or carcinogenicity can be assigned to the growing list of analytes that researchers are now able to detect. This is especially true for the APAHs which, in oils and uncombusted fuels, represent the majority of PAH contaminants resulting from environmental spills and accidents (Baird et al. 2007, Saha et al. 2009, Xia et al. 2012). How then do we deal with more sensitive and comprehensive results and the increased concerns that they may elicit in the general public? Should monitoring efforts simply avoid evaluating the levels of this growing list of PAHs, including APAHs, until they can effectively be used in policy-based, public health protection and management strategies?

Conclusions

The questions and issues raised in this commentary represent deficiencies that the entire cadre of stakeholders including the affected public, research scientists, public health officials, medical professionals, funding agencies and industries can help address. Investment is needed in both research and education to fill the gaps we have identified in critical knowledge and potentially public perception. This group posits that investments in environmental education and literacy will give these stakeholders a better understanding of why an expanded list of PAH analytes, including APAHs is warranted. Additionally, the stakeholders will better understand what the various quantitative levels of PAHs mean from a point of evidence-based public health protection and gain a greater appreciation for the value of the health risk assessment process including its limitations. Achieving these end results will better engage affected communities, improve the application and use of resources in the areas most impacted, and foster more

effective risk communication and information dissemination strategies. These goals can be achieved with investments in improving analytical chemical methods, toxicology, and risk assessment research to develop the evidence base required to objectively evaluate relevant PAHs, including the APAHs, in the health risk assessment process. Currently, there are both government and academic laboratories with the requisite expertise necessary to perform the critical experimentation required to assign toxicity and related risk values to the relevant compounds (e.g. studying bay and fjord region APAHs to evaluate mutagenicity and mechanistic differences relative to their respective UPAHs). High-throughput screening methods (e.g. *in vitro* reporters or cell systems, the Tox21 initiative) could be used to identify relevant compounds for more detailed experimentation and analysis (e.g. animal toxicity and carcinogenicity using defined mixtures). Scaling or potency factors as well as risk values for defined mixtures themselves can be developed from this work (EPA 2000, EPA 2010). Time and resource requirements will no doubt be substantial. However, this should be a major topic of discussion with relevance to not only PAHs but other large classes of chemical compounds that largely remain untested for toxicity. We cannot test each and every PAH independently and in the exponential number of combinations and concentrations possible. Environmental analyses determining the detectable levels and types of PAHs present in a medium of concern should be used to prioritize testing. Those APAHs of highest abundance in the media of concern should receive higher priority ranking. Furthermore, those APAHs whose parent compound or UPAH is carcinogenic should receive high priority with respect to toxicology testing designed to address cancer risks. Specifically testing those APAHs in which the alkyl side groups modify the characteristics of a bay or fjord region should be considered a priority. We have only briefly mentioned some general approaches above that should be part of the conversation. The National

Toxicology Program takes a number of approaches designed to determine adverse effects caused by chemicals including genotoxicity and carcinogenicity. These encompass new high-throughput initiatives such as Tox21 to the well-standardized 2-year rodent bioassay (Tice 2013). These should be considered where appropriate. Other *in vitro* methods such as high-throughput aryl hydrocarbon receptor binding assays or the CALUX® assay could be used as screening tools. *In silico* tools will no doubt be helpful, but these digital models require bench-derived data to be most informative. No single method or assay is likely to be sufficient in terms of both accuracy and precision. Finally, well-informed and designed whole organism (e.g. rodent bioassays) experiments will still be necessary as this approach better captures the relevant exposure(s) and dose-responses in bioactivation, detoxification, mutagenesis, genome maintenance/dysregulation and ultimately cancer. One system that may be useful is exemplified in the work by Knecht et al. (2013) using a high-throughput zebrafish system to test for developmental toxicity for a variety of OPAHs (Knecht et al. 2013). Systems such as this that exist for evaluating tumor formation (possibly the *Xiphophorus* fish model) or that can be developed for evaluating tumor formation would be useful for rapid testing (Walter and Kazianis 2001). Care must be taken to ensure that any model system developed for evaluating the carcinogenic potency of APAHs accurately models humans in terms of exposure and dose-response as well as cancer. We contend that *in vitro* and cell-based systems alone do not provide the entire context necessary that whole animal studies provide. Through a concerted effort, toxicologists, biochemists, analytical chemists, health risk researchers, and community-based researchers working with policymakers can provide the evidence base from which to effectively translate key findings into public health protection policy. Such policy implementation will itself require considerable time and debate among all stakeholders. However, having an adequate evidence base in place to inform

policymaking will help to improve and modernize efforts directed towards mitigating public health and economic impacts caused by future petroleum spills.

References

ATSDR. 1995. Toxicology Profile for Polycyclic Aromatic Hydrocarbons. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services. Pp. 1-487.

Albinet A, Leoz-Garziandia E, Budzinski H, ViIlenave E. 2007. Polycyclic aromatic hydrocarbons (PAHs), nitrated PAHs and oxygenated PAHs in ambient air of the Marseilles area (South of France): Concentrations and sources. Sci Total Environ 384(1-3):280-292.

Baird SJS, Bailey EA, Vorhees DJ. 2007. Evaluating human risk from exposure to alkylated PAHs in an aquatic system. Hum Ecol Risk Assess 13:322-338.

Collins JF, Brown JP, Alexeeff GV, Salmon AG. 1998. Potency equivalency factors for some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbon derivatives. Reg Tox Pharm 28:45-54.

Dickey RW. 2012. FDA risk assessment of seafood contamination after the BP oil spill. Environ Health Perspect 120(2):a54-a55.

Durant JL, Busby Jr WF, Lafleur AL, Penman BW, Crespi CL. 1996. Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. Mutat Res Genet Toxicol 371(3-4):123-157.

EPA. 2000. Supplemental Guidance to RAGS: Region 4 Bulletins, Human Health Risk Assessment Bulletins. EPA Region 4, originally published November 1995, United States Environmental Protection Agency. Website version last updated May 2000 (currently under revision). http://www.epa.gov/region4/superfund/programs/riskassess/healtbul.html (accessed 14 August 2012).

EPA. 2010. Development of a relative potency factor (RPF) approach for polycyclic aromatic hydrocarbon (PAH) mixtures (External Review Draft). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-08/012A. http://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=194584

Farwell C, Reddy CM, Peacock E, Nelson RK, Washburn L, Valentine DL. 2009. Weathering and the fallout plume of heavy oil from strong petroleum seeps near Coal Oil Point, CA. Environ Sci Tech 43:3542-3548.

FDA (Food and Drug Administration). 2010. Protocol for Interpretation and Use of Sensory Testing and Analytical Chemistry Results for Re-Opening Oil-Impacted Areas Closed to Seafood Harvesting Due to the Deepwater Horizon Oil Spill. http://www.fda.gov/food/ucm217601.htm (accessed 14 August 2012).

Gohlke JM, Doke D, Tipre M, Leader M, Fitzgerald T. 2011. A review of seafood safety after the Deepwater Horizon blowout. Environ Health Perspect 119(8):1062-1069.

Goldstein BD, Osofsky HJ, Lichtveld MY. 2011. The Gulf oil spill. NEJM 364(14):1334-1348.

Hecht SS, Loy M, Mazzarese R, Hoffmann D. 1978. Study of chemical carcinogenesis. 7. Synthesis and mutagenicity of modified chrysenes related to the carcinogen, 5-methylchrysene. J Med Chem 21(1):38-44.

IARC. 1973 (updated 1998). Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds. Volume 3. International Agency for Research on Cancer , World Health Organization. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Pp. 1-18.

Klaunig JE, Kamendulis LM. 2008. Chemical carcinogenesis. In: Casarett and Doull's Toxicology: The Basic Science of Poisons (Klaasen CD, ed.) New York: McGraw-Hill, 329-380.

Knecht AL, Goodale BC, Truong L, Simonich MT, Swanson AJ, Matzke MM, et al. 2013. Comparative developmental toxicity of environmentally relevant oxygenated PAHs. Toxicol Appl Pharmacol; http://dx.doi.org/10.1016/j.taap.2013.05.006 [Online 14 May 2013].

Lundstedt S, White PA, Lemieux CL, Lynes KD, Lambert IB, Oberg L, et al. 2007. Sources, fate, and toxic hazards of oxygenated polycyclic aromatic hydrocarbons (PAHs) at PAH-contaminated sites. Ambio 36(6):475-485.

NTP. 2011. Report on Carcinogens. 12th Edition. National Toxicology Program, U.S. Department of Health and Human Services. Pp. 1-507.

Nisbet IC, LaGoy PK. 1992. Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). Reg Tox Pharm 16:290-300.

Overton EB, Ashton BM, Miles MS. 2004. Historical polycyclic aromatic and petrogenic hydrocarbon loading in Northern Central Gulf of Mexico shelf sediments. Mar Poll Bull 49(7-8):557-563.

Peacock N, Field LJ. 1999. The March 1989 Exxon Valdez oil spill: A case study in responding to subsistence seafood safety issues. In: Evaluating and Communicating Subsistence Seafood Safety in a Cross-cultural Context: Lessons Learned from the Exxon Valdez Oil Spill (Field LJ, Fall JA, Nighswander TS, Peacock N, Varanasi U, eds.) Pensacola, FL: Society of Environmental Toxicology and Chemistry, 1-19.

Rotkin-Ellman M, Wong KK, Solomon GM. 2011. Seafood contamination after the BP Gulf oil spill and risks to vulnerable populations: A critique of the FDA risk assessment. Environ Health Perspect 120(2):157-161.

Rotkin-Ellman M, Solomon G. 2012. FDA risk assessment of seafood contamination after the BP oil spill: Rotkin-Ellman and Solomon respond. Environ Health Perspect 120(2):a55-a56.

Saha M, Togo A, Mizukawa K, Murakami M, Takada H, Zakaria MP, et al. 2009. Sources of sedimentary PAHs in tropical Asian waters: Differentiation between pyrogenic and petrogenic sources by alkyl homolog abundance. Mar Poll Bull 58(2):189-200.

Schoeny R., Poirier K. 1993. Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons. United States Environmental Protection Agency, Office of Research and Development. July, 1993. Washington DC. EPA/600/R-93/089. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=49732#Download

Shimada T, Fujii-Kuriyama Y. 2004. Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and1B1. Cancer Science 95(1):1-6.

Silva BO, Adetunde OT, Oluseyi TO, Olayinka KO, Alo BI. 2011. Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria. African J Food Sci 5(7):384-391.

Stolyhwo A, Sikorski ZE. 2005. Polycyclic aromatic hydrocarbons in smoked fish – a critical review. Food Chem 91:303-311.

Tice RR. 2013. Improving the human hazard characterization of chemicals: a Tox21 update. Environ Health Perspect 121(7):756-765.

Walter RB, Kazianis S. 2001. *Xiphophorus* interspecies hybrids as genetic models of induced neoplasia. ILAR Journal 42(4):299-321.

Xia K, Hagood G, Childers C, Atkins J, Rogers B, Ware L, et al. 2012. Polycyclic aromatic hydrocarbons (PAHs) in Mississippi seafood from areas affected by the Deepwater Horizon oil spill. Environ Sci Tech 46(10):5310-5318.